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INFLUENCE OF LH-RF ON LH AND FSH RELEASES IN DOMESTIC MAMMALS

J. PELLETIER

Station de Physiologie de la Reproduction,
Centre de Recherches de Tours, I. N. R. A.,
Nouzilly, 37380 Monnaie (France)

SUMMARY

Intravenous injection of LH-RF into intact rams induces LH release into the blood ; this is related to dose. No clear relationship, however, is observed for FSH. LH peak levels appear after a long time (about 2 hours after a 100 µg synthetic LH-RF injection) ; the maximum level is about 10 ng/ml. Conversely, LH-RF injection given to castrated rams has a rapid effect (10-20 mn), and the peak level is much higher then in intact rams (70-80 ng/ml). This effect is observed as early as 3 hours post-castration. In castrated rams, acute testosterone propionate treatment, given 6 hours before LH-RF, decreases the intensity of LH release by 40 p. 100, but does not modify the time needed to reach peak level. On the other hand, chronic testosterone propionate treatment delays the peak level but, in the conditions used, does not modify the magnitude of the LH peak. Thus, there is may be two different ways in which testosterone acts on LH release through LH-RF action : 1) it decreases LH release after LH-RF ; 2) it delays LH peak time. In the male lamb, LH-RF injection induces LH patterns which vary with age. At 20-60 days of age, LH response to LH-RF is immediate, as in castrates ; thereafter, there is progressive lengthening of this time, which reaches peak level in the intact adult at 140 days of age. Thus, a kind of maturity of the hypothalamohypophyseal system seems to be achieved only at puberty.

In the ewe, intensity of LH release after LH-RF varies during the estrous cycle according to the plasma 17β-oestradiol/progesterone ratio, maximum LH release being observed at day 16 (100 p. 100) and minimum release at day 12 (13.5 p. 100). During seasonal anestrus, LH release after LH-RF also varies according to physiological state : in dry ewes LH release is greater (about 40 p. 100) than in *post partum* lactating or non-lactating ewes.

Influence of LH-RF on LH and FSH releases in other species is discussed in comparison with the above results.

INTRODUCTION

The determination of the pig Luteinizing Hormone Releasing Factor (LH-RF) sequence (MATSUO *et al.*, 1971) opened a new perspective in the hypothalamo-hypophyseal relationship since SCHALLY's group, almost simultaneously with the achievement of LH-RF purification, presented two statements of utmost importance :

first, that LH-RF could be involved in both LH and FSH releases (then called LH-RH/FSH-RH) (SCHALLY *et al.*, 1971), and second, that gonadal steroids could modify pituitary responsiveness to LH-RF (ARIMURA and SCHALLY, 1971).

Furthermore, LH-RF presented an evident interest in the control of gonadotropin release for both medical and zootechnical purposes. Hence, the LH-RF effects on FSH and LH releases were investigated in a number of species ; among these, domestic animals, and mainly sheep, appeared convenient subjects for both fundamental and practical studies.

This paper, then, will describe LH-RF effect on LH and FSH releases in sheep, cattle and pig, with frequent reference to other well-studied species, such as rat or man, for comparison and generalization of various concepts.

I. — LH-RF EFFECTS IN INTACT MALE

Initial studies in ram were carried out with purified sheep LH-RF given in the carotid artery (AMOSS and GUILLEMIN, 1969). A significant extra LH release occurred as early as 3 mn post-injection, and about 30 mn later, the effect was over. On the contrary, purified TRF was without effect. Injection into the jugular vein of crude acidic extract, equivalent to .5 or 1.0 hypothalamus, was shown to induce similar LH release (PELLETIER, 1971). Purified pig LH-RF was also used to induce LH release in ram, but no correlation with doses was observed (REEVES *et al.*, 1970) in the range of 1 to 27 μg. The same group later injected a larger dose of synthetic LH-RF (250 μg) into the carotid artery, but a very incomplete LH pattern was given for one animal only (ARIMURA *et al.*, 1972). Even so, it was apparent that maximum plasma LH value was not reached 1 hour post-injection. Intravenous injections of LH-RF, ranging from 6.25 μg to 1 600 μg, induced a total LH release (computed from the area under the LH curve) which augmented with increasing LH-RF doses (GALLOWAY, 1973) ; LH peak level increased similarly with the dose, and the time to reach this maximum was long (90-150 mn), even for the low doses administered (fig. 1 *a*). Thus, there is a discrepancy between delays in reaching peak level following purified and synthetic LH-RF injection (see above). It is not clearly understood why crude hypothalamic extracts were so efficient since one hypothalamus, including the median eminence, contains about 10 to 20 ng of LH-RF (PELLETIER, unpublished results). However, significant LH release was observed following a 125 ng intramuscular injection (HOPKINSON *et al.*, 1974). The same authors pointed out that : *a*) FSH release was much less sensitive to LH-RF than LH, and *b*) that with the higher doses of LH-RF tested, the only efficient ones (10 and 50 μg), mean LH release was about twenty times higher than the mean FSH release. In the same samples as those used in GALLOWAY's study (1973), FSH release was also investigated using a highly specific homologous ovine FSH radioimmunoassay (BLANC, unpublished results). The FSH response to LH-RF was highly variable and generally only oscillations were observed around the mean pre-injection level (fig. 2). Sometimes the response was more accentuated and a clear immediate increase, representing three times the starting level, was obtained. However, no dose-response relationship was observed in the range of 6.25-1.600 μg LH-RF (BLANC, unpublished results).

In bull, as in ram, blood LH levels increased when doses of synthetic LH-RF were augmented (ZOLMAN *et al.*, 1973 ; GOLTER *et al.*, 1973 ; SCHAMS *et al.*, 1974). Similarly, time elapsed from injection to peak increased with LH-RF, but became

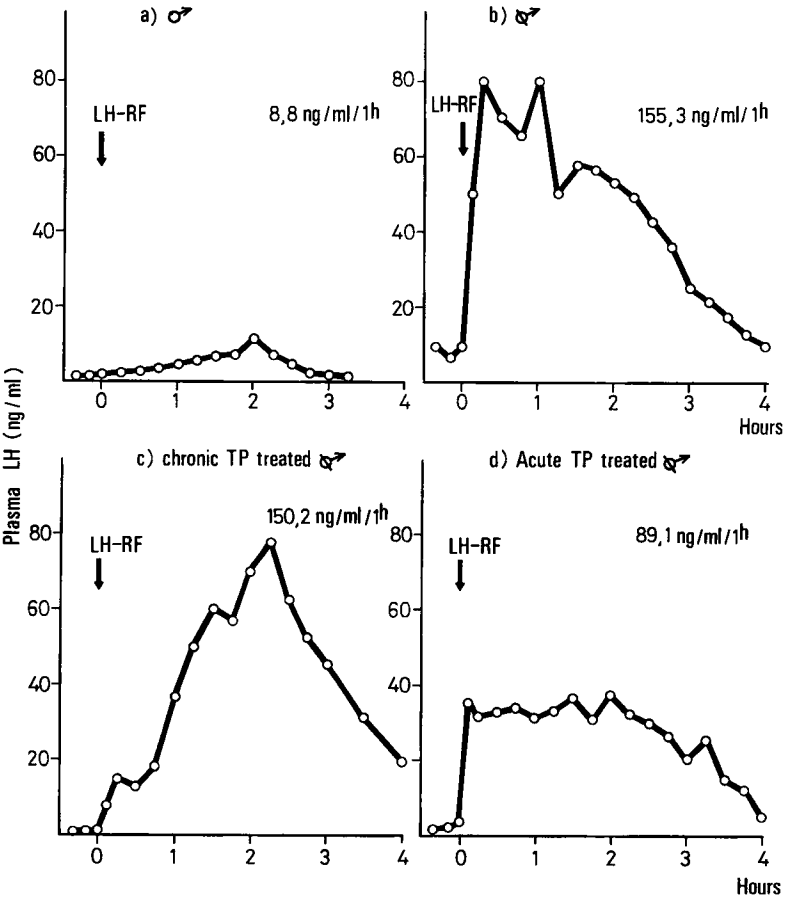

FIG. 1. — *Effect of intravenous injection of 100 µg LH-RF on LH release in :*

a) intact rams ;
b) long-term castrated rams (over 6 months) ;
c) chronic Testosterone Propionate (TP)-treated rams castrated 3 months before (100 mg TP each other day for 14 days) ;
d) Long-term castrated rams treated with LH-RF 6 hours after an intramuscular injection of 600 mg TP (from GALLOWAY, 1973 ; PELLETIER, 1974 *a* ; GALLOWAY and PELLETIER, 1975).

stable above 100 µg LH-RF injection in the 90-140 mn range. At variance with ram, more obvious FSH release occurred following 62.5 to 500 µg LH-RF injections given intramuscularly (SCHAMS *et al.*, 1974), peak FSH level reaching three times the pre-injection level.

In male pig, LH peak was reached 10-15 mn after injection, in the range of 0.05 to 2.0 µg LH-RF/kg body weight, as in the preceding species (ELLENDORFF

et al., 1973 ; POMERANTZ *et al.*, 1974). These LH peaks did not exceed four times the basal level, and thus they differed markedly from those observed in ram and bull.

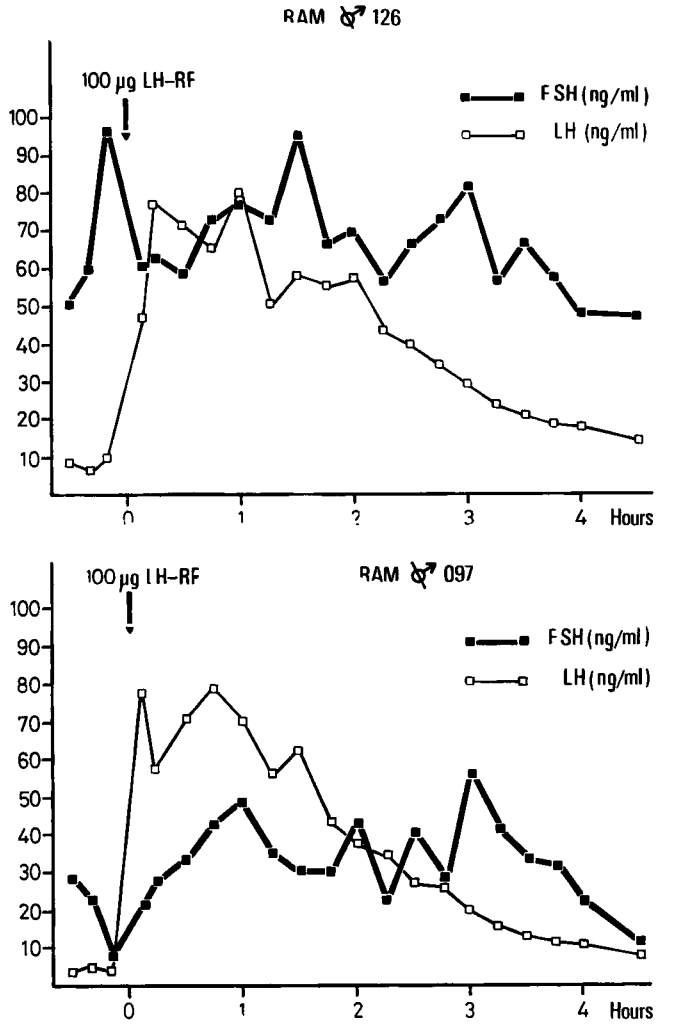

FIG. 2. — *Effect of intravenous injection of 100 µg LH-RF on FSH and LH releases in the castrated ram*

Much more detailed studies were realized in man ; they indicated that intensity of LH response increased with the dose of LH-RF given and that maximum plasma LH level was reached at about 30 mn post-injection (ABE *et al.*, 1972 ; AONO *et al.*, 1973 ; REBAR *et al.*, 1973 ; KLEY *et al.*, 1974 ; FINK *et al.*, 1974 ; YEN *et al.*, 1972 *a*).

Interesting findings resulted from a comparison of LH-RF administration routes. It appeared that intramuscular injections were about as effective as intravenous ones (KELLER and GERBER, 1973), and that the effectiveness of the latter

did not differ from sub-cutaneous injections (ARIMURA *et al.*, 1973). Sublingual or intranasal routes were proven effective, although less efficient, than the three above-mentioned ones (FINK *et al.*, 1974 ; KELLER and GERBER, 1973).

Generally, a more discrete FSH increase took place simultaneously with LH increase after LH-RF administration, but a greater time lapse was frequently needed to reach the peak than in the case of LH (about 60 mn *vs* 30 mn) (AONO *et al.*, 1972 ; ABE *et al.*, 1972 ; YEN *et al.*, 1972 *b* ; REBAR *et al.*, 1973). Very slow and continuous FSH level increase has been shown following LH-RF infusion in man (BREMNER *et al.*, 1974).

Repeated LH-RF treatments were not effective in maintaining high plasma LH level. For example, in entire rams submitted to seven successive 100 μg LH-RF injections at 30 mn intervals, plasma LH rose to a plateau between 90 to 150 mn after the first injection, but the last two treatments failed to prevent a return to pre-injection level (GALLOWAY *et al.*, 1974). Similar findings were shown in different male species such as bull (SCHAMS *et al.*, 1974), rat and hamster (SANDOW and BABEJ, 1973). Plasma FSH seemed differently affected. Repeated treatment did not induce FSH reduction in cattle (SCHAMS *et al.*, 1974). On the other hand, infusion technique, from the point of view of administration route, was considered to improve FSH release in male rats as compared to injection (ARIMURA, DEBELJUK and SCHALLY, 1972 *a* ; DEBELJUK *et al.*, 1973 *a*).

II. — LH-RF EFFECT IN CASTRATED MALES. STEROID INFLUENCE

When purified porcine LH-RF was given to wethers, much stronger LH response was elicited (about ten times the entire male LH response), and the effect was dose-related (REEVES *et al.*, 1970). Peak value was reached very quickly in about ten minutes. Similar results were obtained by GALLOWAY and PELLETIER (1975) in *Ile-de-France* and *Préalpes du Sud* breeds, castrated six months before receiving synthetic LH-RF (fig. 2 *b*). However, the time required to reach peak level after LH-RF differed between breeds (29 ± 5 *vs* 11 ± 2 mn ($P < 0.01$) for the *Ile-de-France* and *Préalpes du Sud*, respectively). The significance of this fact remains to be determined. Very frequently a biphasic pattern in LH release was observed, as in figure 1 *b*, a second peak occurring approximately 1 hour after the first one. It is suggested that in castrated animals where both LH synthesis and release are high, newly synthesized LH could reinforce the intensity of LH secretion from the realizable LH pool.

LH response to LH-RF was also higher in castrated bulls than in entire ones, as far as peak level was concerned. However, the area under the LH curve did not differ significantly (MONGKONPUNYA *et al.*, 1974). Similarly, increased LH release was found in castrated rats treated with LH-RF (WATSON *et al.*, 1971 ; ROOT *et al.*, 1973 ; DEBELJUK *et al.*, 1973 *b*) or in hypogonadic human males (KASTIN *et al.*, 1972 ; ROTH *et al.*, 1972) ; release, in each case, was compared to that in entire males.

Although extra FSH release after LH-RF has been observed in humans and rats, no clear incidence of castration status has been mentioned (WATSON *et al.*, 1971 ; ROOT *et al.*, 1973).

Finally, castrated male LH response to LH-FR differs, at least in rams, from entire male response in two ways. First, the magnitude of the peak level is much greater in the former ; second, delay in reaching the peak is markedly shorter (see fig. 1 *a* and 1 *b*). It may be that the quantitatively greater gonadotropic response to LH-RF is due to higher hormonal pituitary content. To check this point, LH-RF was given to rams which were castrated three hours beforehand under local anesthesia. Figure 3 indicates that the LH response to LH-RF did not differ quantita-

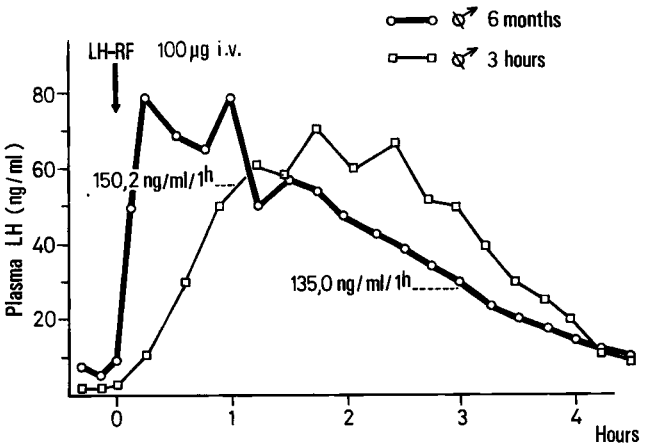

FIG. 3. — *Effect of intravenous injection of 100 µg LH-RF in short-term* (3 hours) *or long-term* (6 months) *castrated rams*
(from GALLOWAY and PELLETIER, 1975)

tively in short-term and long-term castrated rams, although the delay in reaching peak level was different (GALLOWAY and PELLETIER, 1975). This indicates that enhanced response to LH-RF in castrated animals is not due to higher pituitary content, even if it is presumably involved in long-term castrates ; it strongly suggests that the circulating testosterone level ensures a potent inhibitory action on the LH-RF-LH system at pituitary level. Indeed, it requires several months after castration before pituitary gonadotropin content increases in rams (PELLETIER, 1968), but three hours are probably sufficient for testosterone, the main gonadal circulating steroid in this species (ATTAL, 1970), to decrease to a very low level since half-life is short (in the minute range) (TERQUI *et al.*, 1974). Such a striking difference between intact and short-term castrated bulls was not observed by MONGKONPUNYA *et al.* (1974), when LH-RF was given seven days post-castration. The dose used (40 µg) was perhaps too low to fully reveal the difference in sensitivity to LH-RF between entire animals and castrated ones. POMERANTZ *et al.* (1974) found that LH response to LH-RF was greater in castrated than in entire pigs, but that the slopes of the computed log-dose-response curves were not significantly different ($b = 3.22$ for castrates, and $b = 2.20$ for intact pigs). Consequently, the authors rejected the hypothesis of increased pituitary responsiveness to LH-RF in castrates ; they thought that the difference in response to LH-RF could be due to initial pituitary content in castrated and intact animals. However, the three

doses used could be insufficient to accurately describe the LH response curve to LH-RF, and further experiments on short-term castrated animals appear necessary before definitive conclusions can be drawn.

Another approach in studying the feedback effect of gonadal steroids in the hypothalamo-hypophyseal system was to inject testosterone or testosterone propionate into castrated animals.

Acute or chronic treatments produced different results, the quantity of testosterone administered greatly influencing LH response to LH-RF.

In long-term castrated rams, relatively large doses of testosterone were needed to modify plasma LH (PELLETIER, 1970), and this effect was shown to be modulated by light photoperiod (PELLETIER, 1971 ; PELLETIER and ORTAVANT, 1975). Occasionally in June, 200 mg testosterone propionate were insufficient to modify plasma LH (PELLETIER, unpublished results) and a standard dose of 600 mg testosterone or testosterone propionate was adopted. This intramuscular injection induced a

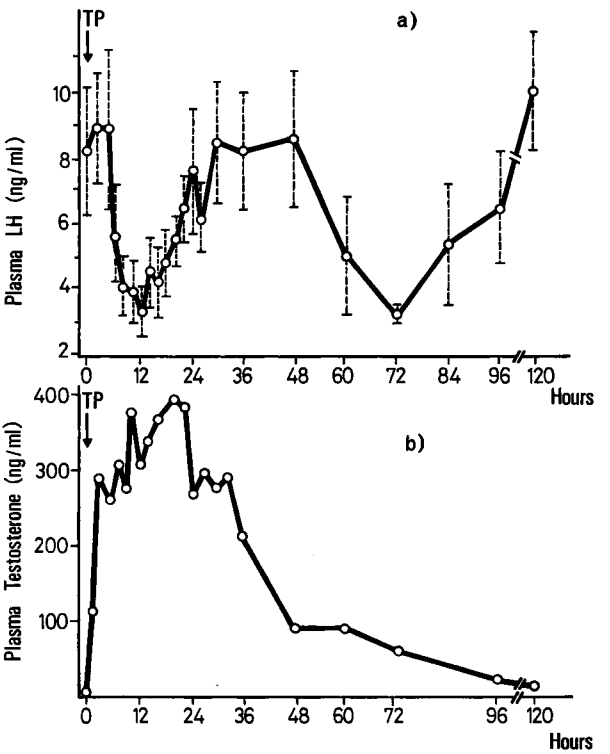

FIG. 4. — *Influences of intramuscular injection of 600 mg testosterone propionate in castrated rams on :*

a) LH release into the blood
b) plasma testosterone level

(From GARNIER *et al.*)

biphasic decrease in plasma LH which was maximum 12 and 72 hours after injection (fig. 4 *a*), these two decreases being separated by a return to the pre-injection level (PELLETIER and ORTAVANT, 1972 ; PELLETIER, 1974 *a*). Compared to the LH res-

ponse to LH-RF observed in castrated control animals, the response was quantitatively similar in rams receiving 100 μg LH-RF, 12, 72 and 120 hours after the testosterone propionate injection. Conversely, LH response was significantly reduced in rams receiving LH-RF 6 hours after the steroid injection (96.0 ± 5.46 *vs* 150.3 ± 2.95 ng/ml/1 h, $P < 0.001$; PELLETIER, 1974 *a*) at a time when plasma testosterone was close to maximum value (fig. 4 *b*) (GARNIER *et al.*). Finally, a decrease in LH response to LH-RF, assumed bo be a decrease in the pituitary responsiveness to LH-RF due to high levels of circulating testosterone, was observed only 6 hours after the steroid injection. The very high circulating testosterone levels needed to modify the pituitary response to LH-RF suggests that in long-term castrated animals, a loss of pituitary sensitivity to testosterone has occurred. However, another possibility is that other steroids, such as oestradiol, participate in the feedback mechanism (see below). Return to pre-injection LH level occurred while plasma testosterone levels were still high ; this suggests that when pituitary is exposed to too long high testosterone levels, it escapes from the inhibitory action of testosterone, as if some preservative mechanism acted as a switch (GARNIER *et al.*). Comparisons of figure 4 *a* and 4 *b* show that this second LH decrease occurs when plasma testosterone itself has been considerably decreased. From concomitant measurements of plasma and pituitary LH and hypothalamic LH-RF activity, it was shown that this second phase of low LH release resulted from a decrease in LH-RF synthesis (PELLETIER, 1970 *b*). Thus, acute strong treatment with testosterone induces a biphasic decrease in plasma LH in castrated rams. This is due to two distinct mechanisms of different latency. Such results have not been accurately observed in entire rams or even in other species where testosterone-LH-LH-RF interrelationships have been studied less extensively. HOPKINSON *et al.* (1974) just mentioned that testosterone propionate decreased LH response to LH-RF in rams.

In intact male dogs, 25 mg testosterone or 2.5 mg dihydrotestosterone were not able to decrease LH response to LH-RF (JONES and BOYNS, 1974). However, the steroid was injected intramuscularly just prior to LH-RF administration. DEBELJUK *et al.* (1972 *a*, 1974) found that both testosterone and dihydrotestosterone, given 48 hours before, depressed, LH-RF action on LH release in castrated male rats. As in the case of intact male rats, these steroids did not modify FSH response to LH-RF.

LH-RF has not been given to rams pre-treated with oestradiol, but it is known that this steroid is highly efficient in decreasing plasma LH (BOLT, 1971), even in long-term castrated males (PELLETIER, unpublished results). For example, 100 μg oestradiol benzoate induced 50 p. 100 LH decrease during the first 12 hours post-treatment, as it did in castrated females. Thus, oestradiol would be about 6 000 times more potent than testosterone in decreasing LH release. Similarly, 17 β-oestradiol or oestradiol benzoate decreased LH response to LH-RF in intact dogs (JONES and BOYNS, 1974) and rats castrated one day before (DEBELJUK *et al.*, 1973 *b*). The combination of testosterone propionate plus oestradiol benzoate strongly inhibited the effect of LH-RF on LH release (DEBELJUK *et al.*, 1972).

VAN DIETEN *et al.* (1974) have shown that oestradiol first inhibited LH response to LH-RF in male rats, but that later, increased response was observed. A similar increase in LH-RF sensitivity, following a period of decreased sensitivity, was also shown in testosterone propionate-treated rams (PELLETIER, 1974 *a*).

The suggestion that other gonadal hormones may be involved in regulating LH secretion (GAY and DEVER, 1971) is supported by the fact that oestrogens are normally secreted by the testis in human, simian and dog (KELCH *et al.*, 1972). If this is true in ram, it would provide a satisfactory hypothesis to explain why so large a quantity of testosterone is needed to decrease plasma LH level. However, no change of testosterone into oestrogens has been observed at peripheral level in testosterone propionate-treated ram (such a change can occur at hypothalamic or pituitary sites), and the hypothesis of progressive loss of pituitary sensitivity to steroids must not be discarded since insensitivity increases with time after castration (PELLETIER, 1970 *a*). This does not agree with the statement that testosterone (and related compounds) absence from the blood, is the only factor responsible for hypersensitivity to LH-RF in castration status.

To summarize, it appears that, when sufficient steroid dose is given at an adequate time before LH-RF administration, a decrease in pituitary responsiveness to LH-RF occurs, at least as far as LH is concerned. But, it is apparent from figure 1 *d*, that this decreased response is not accompanied by an increase in the time required to reach peak LH value after LH-RF administration.

Chronic testosterone propionate treatment of long-term castrated rams (seven successive injections of 200 mg testosterone propionate 48 hours apart) gave a clearly different pattern of LH response to LH-RF than that observed in acute experiments. LH response to LH-RF (given 24 hours after the last steroid injection) was quantitatively similar to that observed in castrated control rams, in spite of low LH level at the time of LH-RF injection (155.1 $\pm$ 23.0 *vs* 147.2 $\pm$ 2.9 ng/ml/1 h ; GALLOWAY and PELLETIER, 1975). However, the time needed to reach peak level was about two hours (fig. 1 *c*), which is very close to that observed in intact LH-RF-treated rams. Thus, in these chronic-treated animals, an increase in the time needed to reach peak level was obtained without any change in response intensity. This result is complementary to that found following acute treatment (compare fig. 1 *b* and 1 *d*) and suggests that in ram, testosterone could act in two different, relatively independent, ways at pituitary level :

a) by inhibiting the magnitude of pituitary response to LH-RF,
b) by delaying LH response to LH-RF.

We have previously seen that quantitative inhibitory action disappeared first, quickly after castration (GALLOWAY and PELLETIER, 1975).

After a chronic testosterone treatment for six days in castrated cattle, MONGKONPUNYA *et al.* (1974) concluded from LH-RF studies that testosterone is not the gonadal factor modifying pituitary responsiveness to LH-RF in bulls. While this assumption is plausible (see above), it cannot be considered conclusive since the actual testosterone treatment (60 mg/day) might have been insufficient in castrated cattle and the influence of the time « last steroid injection-LH-RF administration », which is fundamental to understanding the very effect of LH-RF (PELLETIER, 1974 *a*), has not been studied.

Finally, a natural variation in LH-RF sensitivity, at least in sheep, summarized the changes which occurred when castrated adult rams were compared to chronic testosterone-treated rams : the lamb from birth to puberty. Figure 5 shows that LH-RF, when given at 60 days of age or earlier, induced LH response presenting

a peak level as early as 10-25 mn post-injection. This time delay, a castration-type response, increased with age to reach an adult pattern at 140 days of age ; peak level was reached in about 2 hours (GALLOWAY and PELLETIER, 1974). When two months old, lambs have testosterone in the blood (ATTAL, 1970) and respond to

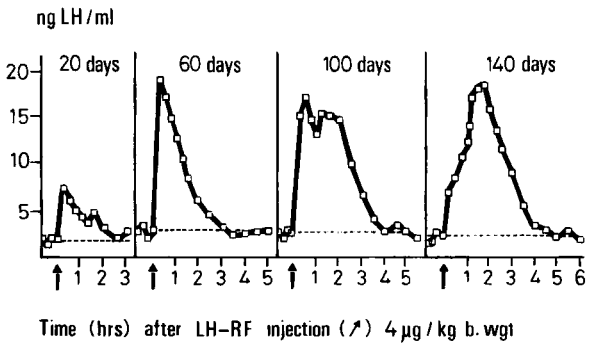

FIG. 5. — *Effect of intravenous injection of LH-RF on LH release in lambs 20, 60, 100 or 140 days old. Note the increase of the delay to reach the peak value of plasma LH with age*
(from GALLOWAY and PELLETIER, 1974)

castration by LH increase (FOSTER *et al.*, 1972), but it would seem that maturity is not achieved, as far as hypothalamo-hypophyseal sensitivity to testosterone is concerned. This is supported by parallel increases in plasma LH and testosterone from birth to week 10 of life (COTTA *et al.*, 1975).

III. — LH-RF EFFECT ON FEMALE LH AND FSH RELEASES

As in the male, the earliest studies conducted in ewe indicated that crude ovine hypothalamic extract injections induced immediates LH release into the blood, the maximum value reaching about 25 times the pre-injection level in 10-15 mn (NISWENDER, 1969 ; GAY *et al.*, 1970). The intensity of response appeared dose-related. Comparable results were obtained when purified porcine hypothalamic extracts were used (REEVES *et al.*, 1970), although no dose-response curve was found, presumably because doses injected were too low. Later, the availability of synthetic LH-RF encouraged great progress in this field.

Administration of synthetic LH-RF to anestrous ewes induced an immediate increase in plasma FSH and LH levels, and the magnitude of response was related to LH-RF dose ranging from 0.2 to about 500 µg (RIPPEL *et al.*, 1974 ; SYMONS *et al.*, 1974). Peak values were reached later (100-150 mn) when the highest doses were given, but these did not prolong the high-level period, and at about 6 hours post-injection, plasma concentrations were close to baseline. No biphasic pattern in LH release, such as those pointed out by ARIMURA *et al.* (1972), have been mentioned by others.

LH and FSH release patterns are similar. However, while the LH peak value may represent 100 times the pre-injection level, that of FSH is more discrete and

is only five times greater than the baseline (SYMONS *et al.*, 1974). It should be noted that the injection route (intravenous, intramuscular or subcutaneous) seems of little importance in response intensity or the time needed to reach the peak.

When two LH-RF injections were given, the effect was related to the interval between them. If the interval was only 3 hours, FSH and LH responses were rather similar after the two injections, but if it was 24 hours, the LH response was considerably lower (REEVES *et al.*, 1972 ; SYMONS *et al.*, 1974). A third injection 24 hours later was followed by further decreased response.

Similarly, anestrous ewes infused for a period of 24 hours with LH-RF presented an increase in plasma LH level which was maximum 3 hours after the beginning of infusion, and then declined (CHAKRABORTY *et al.*, 1974). However, if the infusion time was only 3 hours, no decline in plasma gonadotropins was apparent and plasma FSH and LH patterns were parallel (JONAS *et al.*, 1973). As in the male, these results suggest a change in the pituitary responsiveness to LH-RF due to steroids.

The effects of LH-RF administered to heifers are similar to those observed in ewe, both for the relative increases of plasma FSH and LH levels and the time needed to reach the peak (ZOLMAN *et al.*, 1973 ; KALTENBACH *et al.*, 1974 ; SCHAMS *et al.*, 1974). Repeated treatments with LH-RF are followed by a decrease in LH response, but, rather curiously, that of FSH does not (SCHAMS *et al.*, 1974).

From the limited data available, it appears that in pig, LH response to 25 µg LH-RF was immediate but low (CHAKRABORTY *et al.*, 1973), a fact which may be related to weak preovulatory surge (NISWENDER *et al.*, 1970) rather than to the relatively small dose used since increasing the amount of LH-RF administered up to 1 mg did not improve LH release (BAKER *et al.*, 1973). The LH response to LH-RF decreased during a chronic treatment at 6 hour intervals, but was not abolished (CHAKRABORTY *et al.*, 1973).

IV. — VARIATIONS IN PITUITARY RESPONSIVENESS TO LH-RF IN THE FEMALE. STEROID INFLUENCE

In 1964, BOGDANOVE suggested that variation of LH release into the blood could be the result of a change of pituitary responsiveness to hypothalamic hormone. Six years later, REEVES *et al.* (1971 *b*) showed that LH response to purified porcine hypothalamus injected into ewes varied during the estrous cycle and was maximum around the period of estrus. Furthermore, injecting oestradiol benzoate to anestrous ewes greatly improved LH reponse to LH-RF, an effect again attributed to an incrase in pituitary responsiveness to LH-RF (REEVES *et al.*, 1971 *b*). After these pioneer works, attention was focused, in many species, on intensity of LH release after LH-RF was given at different stages of the estrous cycle or in females pretreated with steroids, oestrogens or progesterone.

Conflicting results were obtained when LH-RF was given at different stages of ewe estrous cycle. Contrary to REEVES *et al.* (1970), FOSTER and CRIGHTON (1974), SYMONS *et al.* (1974) and RIPPEL *et al.* (1974) did not find a difference in LH release intensity after administration of LH-RF during the estrous cycle, but these authors may not have chosen the most representative moments of great steroid variation.

On the other hand, for both FSH and LH, responses to LH-RF were found to be greater in ewes infused for 4 hours on days 15-17 than in those infused on days 2-12 (HOOLEY *et al.*, 1974).

To clear up this problem, we injected *Ile-de-France* ewes with 25 μg of synthetic LH-RF at days 4, 8, 12 or 16 of the estrous cycle, and determined 17β-oestradiol and progesterone plasma levels before LH-RF injection. For each of these four stages of the cycle, 17β-oestradiol was 21, 31, 11 and 31 pg/ml (mean for 3 ewes) and progesterone was 0.80, 2.40, 1.90 and 0.95 ng/ml. Expressed in percentage of the response observed on day 16, the mean LH response to LH-RF was 39, 16 and 13.5 p. 100 for days 4, 8 and 12, respectively. Intensity of LH response was negatively correlated with plasma progesterone level ($r = -0.7$), but a better correlation was obtained between oestradiol/progesterone ratio and LH response to LH-RF ($r = +0.97$, $P < 0.05$) (THIMONIER *et al.*, 1974). Thus, circulating steroids would produce a regulation of LH-RF effect acting at the pituitary level at any time. Similar conclusions have been drawn in ram where the intensity of LH release after LH-RF was correlated with previous circulating testosterone level (GALLOWAY *et al.*, 1974).

Few data are available on other domestic species. ZOLMAN *et al.* (1974) concluded from LH-RF injections to cyclic heifers that the day of treatment in the cycle (15 or 20) did not influence LH response to LH-RF, although an increase in plasma oestradiol and a decrease in plasma progesterone have been registered at D20. However, a more complete study would be interesting.

On the contrary, greater LH response to LH-RF was observed in cows with cystic follicles than in luteal cows, although no differences in oestradiol plasma levels were shown before injection (KITTOK *et al.*, 1973).

Finally, in spite of contradictory results, the hypothesis of a variation in pituitary response to LH-RF according to the stage of the estrous cycle is the most plausible hypothesis in domestic animals. The maximum response would be obtained at times when plasma oestrogens would be high and progesterone level low. Conversely, when the progesterone level is high (for example, during pregnancy) both FSH and LH responses to LH-RF are reduced (CHAMLEY *et al.*, 1974 *a* and *b*). Similar studies in a number of species, such as hamster (ARIMURA *et al.*, 1972 *b*), rat (COOPER *et al.*, 1973 ; AIYER *et al.*, 1974) or man (YEN *et al.*, 1972 *b*), add further evidence for changes in pituitary responsiveness during the cycle. However, the relationship between plasma steroid level and LH response to LH-RF needs further research.

The previous conclusion is supported by works involving steroid administration. REEVES *et al.* (1971 *b*) indicated that benzoate oestradiol injection increased LH response to LH-RF in anestrous ewe. Conversely daily injection of 20 mg progesterone for 14 days or infusion of 500 μg progesterone/hr for 76 hours significantly suppressed LH and FSH responses to LH-RF (PANT and WARD, 1973 ; HOOLEY *et al.*, 1974). Short progesterone infusion (500 μg/hr for 24 hrs ; CUMMINGS *et al.*, 1972 *a*) or pretreatment with pessaries impregnated with 20 mg fluorogestone acetate (9-fluoro 11α-17-dehydroxypregn 4ene, 3-20-dione 17-acetate, Searle Co. ; CHAKRABORTY *et al.*, 1974) did not modify pituitary response to LH-RF in anestrous ewes. Furthermore, a decrease in pituitary responsiveness also occurred when oestradiol and progesterone were given together (DEBELJUK *et al.*, 1972 *b*).

Increased pituitary responsiveness to LH-RF following oestrogen administration was also shown in female rat (ARIMURA and SCHALLY, 1971) and in woman (JAFFE and KEYE, 1974), but more precise studies indicated in both cases that oestrogens had two opposite effects. First, when given a few hours (4 to 6) before LH-RF, oestrogens decrease LH response to the latter (LIBERTUN *et al.*, 1974 ; VILCHEZ-MARTINEZ *et al.*, 1974 ; LEGAN *et al.*, 1974 ; THOMPSON *et al.*, 1973). From changes in FSH/LH plasma ratio, it would seem that inhibitory effect of oestrogen could affect preferentially the FSH more than the LH release (LIBERTUN *et al.*, 1974 ; KEYE and JAFFE, 1974). Second, an amplified effect of LH-RF is observed if the time between oestrogen and LH-RF treatments increases. When it reaches 24 hours, LH response to LH-RF increases with the pretreatment dose of oestrogen used (KULKARNI *et al.*, 1974). In sheep, similar conclusions can be drawn when one considers plasma LH patterns in ovariectomized ewes injected with 50 μg oestradiol benzoate (PELLETIER and SIGNORET, 1970) : the plasma LH decreased about 80 p. 100 during the first 14 hours post-injection before a « preovulatory » type of surge occurred, and this decline is presumably correlated whith an initial decrease in pituitary responsiveness to LH-RF. In Australian ewes grazing oestrogenic clover, the LH release-inducing effect of exogenous oestradiol is more or less abolished, but LH response to LH-RF infusion is not (FINDLAY *et al.*, 1973). As in sheep, progesterone has been generally accepted as decreasing the LH-RF effect in rat (ARIMURA and SCHALLY, 1970), rabbit (HILLARD *et al.*, 1971) or woman (THOMPSON *et al.*, 1973).

To summarize, oestrogens would have a biphasic effect, first inhibitory then facilitative, on gonadotropic response to LH-RF ; progesterone itself and high concurrent levels of oestrogen and progesterone would, on the contrary, have an inhibitory effect.

V. — LH-RF EFFECTS IN ANESTROUS EWES AND LACTATING EWES

LH-RF has not only been considered as a proven tool for fundamental research ; it focused the attention of workers trying ot induce FSH and LH releases in reproductive animals which have intermittent cyclicity during the year. It was also important to clinicians confronting anovulatory disorders in woman (ZARATE *et al.*, 1972). If we consider reproductive animals only, sheep is mainly involved since the female cyclicity usually stops for several months, depending on the breed and country.

Preovulatory LH release was initially induced in anestrous ewe with either oestradiol (GODING *et al.*, 1969 ; RADFORD *et al.*, 1970 ; BECK and REEVES, 1973) or PMSG (THIMONIER and COGNIE, 1971). However, in ewes synchronized with a progestagen, oestradiol tended to reduce fertility (ROBINSON *et al.*, 1970) and PMSG induced appearance of refractoriness leading to reduced ovulation rate when given too frequently. Thus, LH-RF could be considered as a more convenient inducer of LH release. REEVES *et al.* (1972), JONAS *et al.* (1974), SYMONS *et al.* (1974) induced both LH and FSH releases with LH-RF given to anestrous ewes, and REEVES *et al.* (1974) indicated that intensity of response did not differ in early, mid or late anestrus. We have confirmed that in ewes synchronized with vaginal sponges impregnated with 40 mg FGA, injected with 600 IU PMSG the day of sponge removal

and subjected 24 hours later to two intramuscular injections of 200 μg LH-RF at a four hour interval, intensity of LH release is as great as in ewes during sexual season (PELLETIER, 1974 *b*). However, omission of PMSG injection results in a significant 26 p. 100 decrease in the quantity of LH released, computed from the area between the actual LH pattern and the pre-injection level assumed to be constant. Intensity of LH release was similarly reduced in ewes receiving one 400 μg injection of LH-RH in place of two 200 μg injections with or without PMSG. Our data differ from those of RIPPEL *et al.* (1974), who found that LH release in anestrous ewes receiving only 100 μg LH-RF was as great or even greater than that of cyclic ewes. In the latter case, however, females were not synchronized.

TABLE I

Influence of LH-RF on LH release in dry ewes and in 40 day post partum *lactating and-non lactating ewes treated during seasonal anoestrus*

Group	n	PMSG-LHRF (1) Interval (hours)	LH release (2) (ng/ml/1 h)
Dry ewe	6	24	313 ± 47.0
Dry ewe	6	30	279 ± 44.3
Lactating ewe	6	24	169 ± 30.0
Lactating ewe	6	30	177 ± 47.0
Non-lactating ewe	4	24	141 ± 7.9

(1) All females were synchronized with 40 mg fluorogestone acetate given intravaginally for 6 days and treated immediately after sponge removal with 600 IU (dry ewes) or 750 IU (post-partum ewes) PMGS given intramuscularly and later (24 or 30 hours) with intravenous injections of LH-RF (twice 200 μg at 4 hour interval).

(2) Total LH release was expressed in ng/ml per one hour as if all the LH release occurred in the interval of one hour only (in part from PELLETIER, 1974 *b*).

It was more difficult to induce preovulatory LH release in the post-partum period, namely, when lactating anestrus is superimposed on seasonal anestrus. In these conditions, it has been shown that PMSG or oestradiol LH-induced release was 20 to 30 p. 100 less than that observed in similarly treated dry ewes (PELLETIER and THIMONIER, 1973 ; LEWIS *et al.*, 1974), although the pituitary contents were not found to be different in the two cases (PELLETIER and THIMONIER, unpublished results). Attempts to use LH-RF as an inducer of LH release led to similar results when the lactating females were injected between the third and sixth week post-partum (CHAMLEY *et al.*, 1973 ; RIPPEL *et al.*, 1974). With our above-mentioned conditions, LH release after LH-RF is about 40 p. 100 less in lactating ewes than in dry ewes (table I) (PELLETIER, 1974 *b*). According to JENKIN and HEAP (1974), the lactation itself would not have an effect *per se* since a similar decrease in the pituitary response to LH-RF was also observed when lambs were removed at birth. Our preliminary data (table I) confirm this assumption. The low circulating levels of

oestradiol found in lactating females (COGNIE *et al.*, 1974) suggested that a decrease in pituitary responsiveness could be responsible for this reduced response to LH-RF, and it was interesting to see if increasing the dose of LH-RF would overcome this lack of sensitivity. During the period of deep anestrus, March, dry ewes or lactating females on the 28th day post-partum were first given a synchronization treatment with vaginal sponges impregnated with progestogen (30 mg FGA for 12 days) and a PMSG intramuscular injection (400 IU) at the time of sponge removal. Dry ewes and one group of lactating females then received two injections of 200 µg LH-RF at four-hour intervals 24 hours later. Two other groups of lactating ewes similarly received two injections of 400 and 600 µg LH-RF, respectively. Representative LH patterns for each of the four groups are given in figure 6. The mean LH releases,

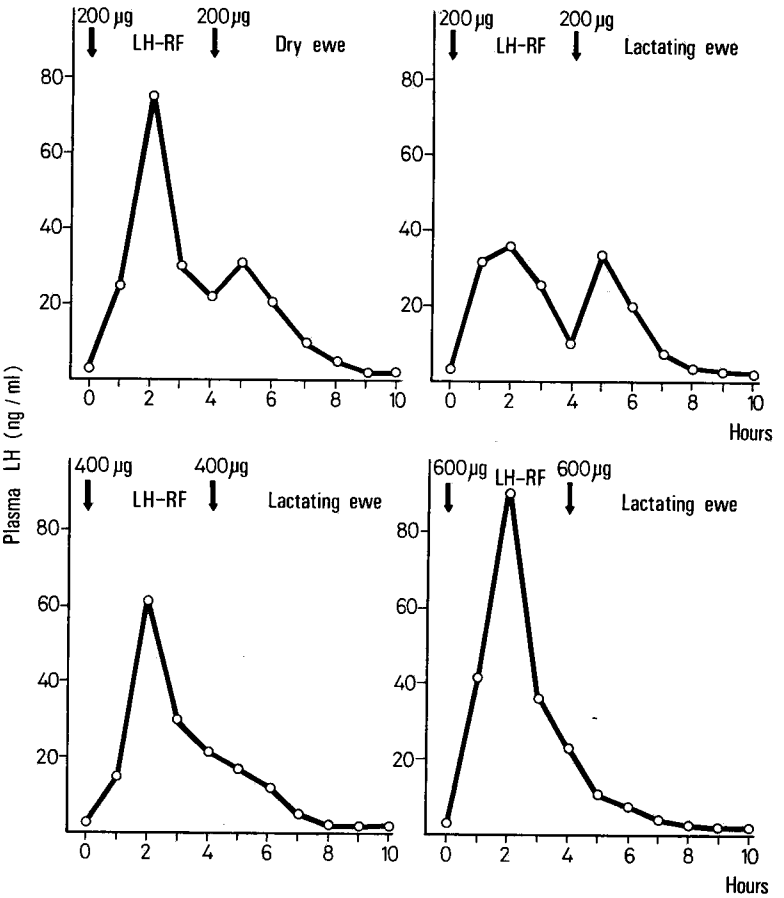

FIG. 6. — *Influence of doses of LH-RF on LH release in lactating ewes* (40 d. *post-partum*) *in comparison with anoestrous dry ewes*

computed from the area under the curve, were 66.4, 73.7 and 100 p. 100 of that observed in dry ewes in the 2 × 200, 2 × 400, 2 × 600 µg LH-RF-treated groups, respectively. Further, as the LH-RF dose increased, so did LH response to the first

injection, but the response to the second injection was progressively abolished. This suggest that pituitary stores of releaseable LH were almost completely depleted and that new LH was resynthesized at slow rates in these conditions. From a practical point of view, massive unique LH-RF injection would be better for the induction of LH release in lactating ewes during the seasonal anestrous period as compared to dry ewes where two injections were fund convenient (REEVES *et al.*, 1972 ; SYMONS *et al.*, 1974).

RIPPEL *et al.* (1974) suggested that decreased pituitary release during lactation was the main factor in infertility. Our feeling is that the hypothalamo-hypophyseal system and genital tract are both involved since ovulation rate and fertility did not appear correlated to intensity of the LH release (THIMONIER and PELLETIER, 1972 ; LAND *et al.*, 1973). However, LH release comparable to preovulatory LH surge of the cyclic ewe is needed in synchronized lactating ewes in order to determine other sources of infertility.

Two other critical points remain open to question. First, in spite of the report of MOSS and MCCANN (1973), showing that estrus behavior could be induced with LH-RF in female rats, no estrus or irregular onset of estrus behavior was shown in LH-RF-treated ewes (SYMONS *et al.*, 1974 ; SEGERSON *et al.*, 1974, and our personal observations). However, if LH-RF, given at an inapropriate time before estrus in cyclic ewe can induce premature ovulation and thus reduce normal fertility (SEGERSON *et al.*, 1974), the incidence of estrus failure *per se* has not been evaluated. Similarly, the lack of estrus could not be relevant to fertility in lactating LH-RF-treated ewes since preovulatory LH releases are perfectly synchronized and theoretically authorize artificial insemination at an exact time just before ovulation. A clear picture of the relationship between the presence of estrus and fertility is highly needed. Second, the relationship between LH-RF-induced LH release and ovulation has to be considered both in the light of incidence of ovulation and quality of ovulation. If anestrous ewes, treated with LH-RF given by perfusion (CHAKRABORTY *et al.*, 1974) or with low acute injection (25 or 100 μg once ; RIPPEL *et al.*, 1974), did not ovulate, females treated with our progesterone-PMSG-LRF (see above) ovulated readily ; the ovulation rate was close to that observed in similarly treated dry ewes (PELLETIER, 1974 *b*). The quality of the ovulating process is a more difficult point. On one hand, the LH release-ovulation interval, assumed to be constant (CUMMING *et al.*, 1972 *b*), could differ by 24 hours in PMSG-superovulated lactating females and in the same animal, ovulations could occur at 30 hour intervals (COGNIE and PELLETIER, in preparation). It si highly questionable whether LH-RF will improve the staggered ovulations. On the other hand, LH-RF induces LH and FSH releases but these releases may not, on the whole, represent qualitatively all the « ovulating » release if : *a*) there is a FSH-RF different from LH-RF, *b*) other pituitary hormones are involved in ovulation.

CONCLUSION

In the last few years, LH-RF has been extensively used, and this paper reviews the large number of results achieved. However, new questions arise and areas of doubt appear. For example why do crude or purified hypothalamic extracts induce

immediate LH response in intact rams in the nanogram range, while maximum LH response to synthetic LH-RF is delayed about two hours and that micrograms are needed ? Furthermore, discrete and frequently erratic FSH responses are observed following LH-RFad ministration ; afterwards, definite conclusions cannot be drawn from the results positively identifying FSH-RF and LH-RF. Another disquieting point is the frequent absence of systematic study of the delay effect between steroid and LH-RF administration when there may be a change in pituitary responsiveness to LH-RF. More detailed studies need to be done on all these points.

From a practical point of view, the use of LH-RF as an inducer of LH release in anestrous ewe must be completed with other hormonal treatments since the status of the genital tract in post-parturient females surely affects fertility. Presumably, a great deal of research will be necessary before such a « cocktail » works with success.

Scientists must re-new their efforts before the effect of synthetic LH-RF availability can really be assessed, especially in domestic animals.

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RÉSUMÉ

INFLUENCE DU LH-RF SUR LA LIBÉRATION DE LH ET DE FSH CHEZ LES ANIMAUX DOMESTIQUES

L'injection intraveineuse de LH-RF à des béliers intacts provoque une libération de LH dans le sang qui est fonction de la dose ; aucune relation n'est observée dans le cas de FSH. Le délai entre l'injection de 100 µg de LH-RF synthétique est long, de l'ordre de 2 heures, et le niveau maximum atteint est d'environ 10 ng/ml de LH. Inversement lorsque le LH-RF est injecté à des béliers castrés le délai pour atteindre le pic est court (10-20 mn) et la valeur du pic est beaucoup plus élevée que chez le bélier intact (70-80 ng/ml). Un tel effet est observé dès 3 heures après la castration. Chez les béliers castrés, une injection unique de 600 mg de propionate de testostérone effectuée 6 heures avant celle du LH-RF diminue le pic de LH de l'ordre de 40 p. 100 sans modifier le temps nécessaire pour atteindre le pic. D'autre part, un traitement chronique par le propionate de testostérone retarde le moment du pic par rapport à l'injection de LH-RF sans modifier l'intensité de la décharge. Il semble donc que la testostérone agisse selon deux voies différentes :

a) en diminuant la réponse de LH au LH-RF ;
b) en retardant le moment du pic LH.

Chez l'Agneau mâle, l'injection de LH-RF induit une décharge de LH variable selon l'âge considéré. A 20-60 jours d'âge, le LH-RF induit une libération de LH immédiate comme chez le bélier castré, puis à des âges plus avancés, on observe une augmentation du délai entre l'injection de LH-RF et le pic de LH. A 140 jours, ce délai est comparable à celui observé chez l'adulte. Ces résultats suggèrent que la maturité du système hypothalamo-hypophysaire n'est achevée qu'à la puberté.

Chez la Brebis, l'intensité de la libération de LH après injection intraveineuse de LH-RF varie au cours du cycle œstrien et est corrélée avec le rapport œstradiol-17β/progestérone plasma-

tiques. Le maximum de libération de LH est obtenu au jour 16 du cycle (100 p. 100) et le minimum au jour 12 (13,5 p. 100). La libération de LH après LH-RF varie aussi en fonction de l'état physiologique : chez la brebis sèche la décharge de LH induite est supérieure d'environ 40 p. 100 à celle observée chez la brebis allaitante ou tarie après parturition.

Ces résultats sont discutés en parallèle avec ceux obtenus chez les autres espèces.

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